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Distribution of Orally Administered Chrysotile Asbestos in Newborn Baboon Body

Illinois Univ. at the Medical Center
Chicago

Prepared for

Health Effects Research Lab.
Cincinnati, OH

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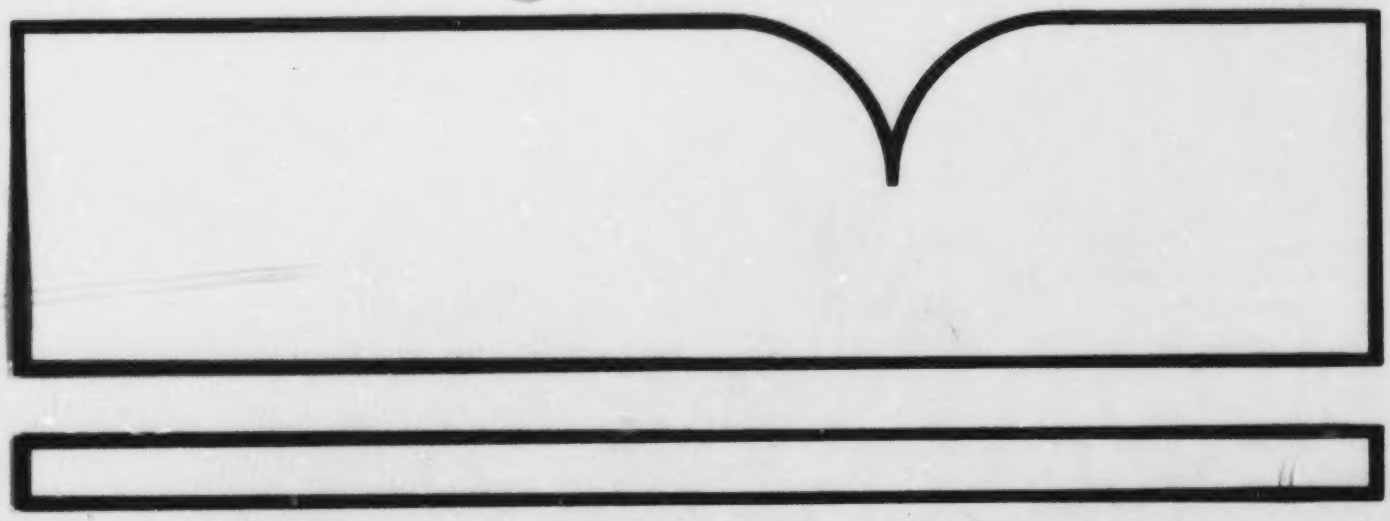

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16. ABSTRACT

This report presents the results of transmission electron microscopic analyses for asbestos in ten organs of the newborn baboon which was orally administered chrysotile asbestos. The feeding period consisted of nine days with a cumulative dose of 3×10^{13} chrysotile fibers per kilogram. Analyses of the same ten organs of a control newborn baboon is also included.

The test tissues showed higher levels of asbestos than respective control tissues. The highest concentration of chrysotile fibers was found in the kidney cortex followed by lymph nodes, spleen, colon, esophagus, kidney medulla, stomach, and liver. Fiber size distribution of the fibers recovered from all organs is presented.

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EPA-600/1-80-022
May 1980

DISTRIBUTION OF ORALLY ADMINISTERED CHRYSOTILE ASBESTOS IN NEWBORN BABOON BODY

by

Kusum J. Patel-Mandlik
School of Public Health
University of Illinois
Chicago, Illinois 60680

Contract No. CA-8-3307-J

Project Officer

James R. Millette
Field Studies Division
Health Effects Research Laboratory
Cincinnati, Ohio 45268

HEALTH EFFECTS RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OHIO 45268

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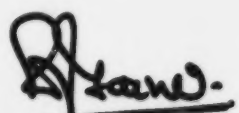
FOREWORD

The U. S. Environmental Protection Agency was created in response to increasing public concern about the dangers of pollution to the health and welfare of the American people and their environment. The complexities of environmental problems originate in the deep interdependent relationships between the various physical and biological segments of man's natural and social world. Solutions to these environmental problems require an integrated program of research and development using input from a number of disciplines.

The Health Effects Research Laboratory was established to provide sound health effects data in support of the regulatory activities of the EPA. Evaluating man's exposure to environmental health hazards is a key segment in developing such a data bank. Studies of exposure require identification, characterization, and quantification of physical, chemical, and biological agents found in the environment. In addition, exposure assessment involves the determination of conditions that cause agents to be released into the environment, the study of the routes and pathways to man, and research into the body's ability to prevent the entrance of environmental hazards.

This report presents the results of a study to determine if ingested asbestos fibers can penetrate the gastrointestinal tract and be transported to other tissues in the body. An understanding of how the body handles asbestos is important in determining the potential adverse health effects of asbestos in drinking water.



R. J. Garner
Director
Health Effects Research Laboratory

ABSTRACT

This report presents the results of transmission electron microscopic analyses for asbestos in ten organs of the newborn baboon which was orally administered chrysotile asbestos. The feeding period consisted of nine days with a cumulative dose of 3×10^{13} chrysotile fibers per kilogram. Analysis of the same ten organs of a control newborn baboon is also included.

The test tissues showed higher levels of asbestos than respective control tissues. The highest concentration of chrysotile fibers was found in the kidney cortex followed by lymph nodes, spleen, colon, esophagus, kidney medulla, stomach, and liver. Fiber size distribution of the fibers recovered from all organs is presented.

A portion of this study appeared in EPA-600/1-78-069. This report was submitted in fulfillment of Contract No. CA-8-3307-J by the School of Public Health, University of Illinois Medical Center, under the sponsorship of the U.S. Environmental Protection Agency. This report covers the period October 1, 1978 to September 30, 1979, and work was completed as of September 10, 1979.

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INTRODUCTION

Exposure to asbestos occurs to small groups of people in their workplaces or to the general population through air, food, water and beverages.[1-6] A few references are cited here from the extensive literature on lung cancer, mesothelioma, and asbestosis occurring from exposure to occupationally inhaled asbestos.[7-10] Hazards of orally-ingested asbestos are not yet understood. Reports have appeared showing that the ingested fibers penetrate, migrate between, and are recoverable from the gastrointestinal tract and distant tissues,[11-13] as well as from urine.[14,15]

An understanding of how the body handles ingested asbestos is important in determining the potential health effects of asbestos in drinking water, foods, drugs, and beverages. This report presents the results of a study to determine if orally-administered asbestos in a nonhuman primate can be recovered from tissues other than the gastrointestinal tract organs and in what concentrations. This is an extension of a previous study wherein recovery of high concentration of asbestos in kidney cortex of a test newborn baboon was reported.[13] Preliminary results of recovery of asbestos in kidney medulla, spleen, and liver of this baboon were given earlier.[16] This report includes more definitive data on kidney medulla, liver, and spleen, and further analyses for asbestos in the gastrointestinal tract organs, heart, liver, and lymph nodes.

CONCLUSIONS

This study was done to investigate the distribution pattern of ingested asbestos in an animal species closely related to humans, the baboon. Newborn control and test baboons *(Papio anubis)*, one each was bottle fed *ad libitum* with Similac milk formula. The milk formula fed to test baboon was mixed with UICC Canadian Standard Reference Chrysotile. Tissues taken from ten different organs of each animal were analyzed for asbestos fibers. Chrysotile asbestos was found in the gastrointestinal tract organs, kidney cortex and medulla, spleen, lymph nodes, and liver of the test baboon; whereas test heart showed a below detection level. Among ten organs of the control neonate baboon, only a very low number of fibers were recovered in each instance except from the cecum. Test kidney cortex and lymph nodes showed highly significant difference from the controls ($P \leq 0.001$). Test kidney cortex had the highest level of fibers among all the organs ($P \leq 0.001$).

Evidence is provided indicative of penetration and migration of orally administered chrysotile asbestos through the gut wall into and between other organs of the body. Also, fibers of wide ranges of lengths and widths seem to penetrate and migrate without a particular selection mechanism.

RECOMMENDATIONS

1. Further research is necessary to elucidate the mechanism by which chrysotile fibers are degraded within the biological system.

2. Further work should be conducted to confirm that kidney is the site of highest retention of ingested asbestos and to investigate the reason(s) and any possible damage caused to the ultrastructure of the kidney.

3. There is a sparsity of data on retention of ingested asbestos by cancer target organs of the body following chronic oral exposure to asbestos. Such information could be useful to correlate it with the results of studies on carcinogenicity of asbestos.

MATERIALS AND METHODS

Two neonate baboons (*Papio anubis*), born exactly 8 days apart, were used as control and test animals. Each animal was maintained in an Isolette infant incubator under conditions of controlled temperature, humidity and air supply. The test animal weighed 920 grams and was bottle fed with a suspension of UICC Canadian chrysotile in milk formula. See Table 3 for the frequency distribution of UICC fiber lengths. The suspension was prepared by swirling 10 mg of asbestos in 500 ml of distilled water which had been previously filtered through a 0.1µm Millipore membrane. It was mixed 1:1 with concentrated milk formula (Similac, Ross Laboratories) prior to feeding to give 10 mg of chrysotile per liter. Assuming 10^6 fibers per nanogram, the feeding suspension contained 10^{13} fibers per liter (f/ℓ). A total of 2.8 liters was provided *ad libitum* over a period of 9 days beginning 24 hours after birth. Therefore, the total dose for the test animal was 3.0×10^{13} F/kg. The control animal weighed 961 g and was bottle fed with a one to one mixture of concentrated milk formula and distilled, filtered water; 2.6 liters were consumed over 9 days. Both animals were sacrificed at the end of the 10-day trial.

TISSUE PREPARATION

1. Comparable weights of control and test tissues were dried in a vacuum oven at 90°C until weights became constant.

2. Dried tissue was ashed in a low temperature plasma asher at 80 watts for about 3 hours.

3. The resultant ash was suspended in 10 ml of 1% acetic acid and ultrasonicated at 50kHz for 1 minute to obtain dispersion without breakage. Treatment with acid improved the removal of organics in the subsequent ashing step. A check using reference UICC Canadian chrysotile indicated that acid treatment did not disturb the morphology or diffraction pattern under transmission electron microscopy.

4. The suspension was filtered through a 0.1µm Nucleopore membrane and the latter was ashed in the plasma unit at 80 watts for 3 hours. The residue was suspended in 5 ml of filtered (0.2µm Fluoropore) acetone and sonicated for one minute.

5. The volume of acetone-tissue ash suspension to be deposited on Formvar and carbon-coated TEM grid depended upon the dry weight of each tissue sample. Rapid drying of the suspension delivered from a microlitre syringe was effected by the use of an infrared lamp (250 W) at four-five inches from the grid. Acetone suspensions were kept covered and

cold to minimize evaporation of acetone during grid preparation.

6. Between samples, the syringe was rinsed several times with filtered acetone. Syringe contamination was not a problem as evidenced by the below-detection results obtained for several of the tissue preparations. It is important to note that test and control tissues were prepared in random sequence.

ELECTRON MICROSCOPIC SCANNING AND CALCULATIONS

The grids were examined at a magnification of 19,000 under TEM Philips 300. Morphology and occasionally selected area electron diffraction (SAED) were used to identify chrysotile asbestos. Fifty grid holes, 25 from each of two grids prepared from same ash suspension, were scanned for each control and test tissue. Fibers recovered in control samples served not only as count of the control tissues, but also as background count of all the materials and procedures used in preparing the grids.

Fibers were counted and sized for length and width. In case of a bundle or cluster, length of the longest fiber and width of the widest region were measured. A bundle or cluster was counted as one fiber. Note that a bundle is defined here as two or more fibrils touching each other along their long axes, and a cluster is that which is composed of two or more individual fibers and/or bundles oriented at various angles to each other, except parallel at any point of contact. The fibers were identified and graded as Class A, Class B, and Class C as follows:

Class A - Fibers which were clearly representative of chrysotile morphology and sometimes SAED.
Class B - Fibers which were somewhat degraded, but still having a morphology of chrysotile.
Class C - Fibers which were degraded and barely identifiable by morphology.

The final concentration of fibers per mg dry weight of tissue was calculated by employing the total of fibers in Class A and Class B recovered in 50 grid holes per tissue. The equation used for calculations was as follows:

$$\text{Fibers/mg dry weight of tissue} = \frac{F \times A_1 \times V_1}{G \times A_2 \times V_2 \times M} \quad \ldots\ldots\ldots\ldots \text{(i)}$$

$$= \frac{F \times 80}{V_2 \times M} \quad \ldots\ldots\ldots\ldots\ldots\ldots \text{(ii)}$$

where F = total number of fibers recovered per tissue
A_1= 0.07 cm^2 = area of a 200 mesh TEM
V_1= 5 ml = volume of ash-acetone grid suspension
G = 50 = total number of grid holes examined per tissue
A_2= 8 X 10^{-5} cm^2 = area of 1 grid hole

V_2= μℓ volume deposited on grid
M = weight of dry tissue in mg

A loading factor, L, was derived to permit quick comparisons of fiber retention (1) between control and test tissues of the same type and (2) among different types of tissues within the test group, without conversion of the EM scanning data to a unit of fiber concentration/mg dry wt. of tissue. L equalled V_2 X M.

By substituting L for V_2 X M in the above equation, it can be rewritten

$$\text{as } \frac{F \times 80}{L} \quad \ldots\ldots\ldots\ldots\ldots\ldots \text{(iii)}$$

The value of L was maintained almost equal in the test and control pairs of tissues, as well as among the tissues by adjusting the volumes of suspensions to be deposited on grids. This was, in other words, to normalize the small variations in dry weights of tissue samples. As shown in Table 1, the values of L for 20 tissue preparations (excluding kidney cortex preparations since they were from a separate study) ranged from 0.89 to 1.086. Since the volume of total suspension, V_1, is five in each case, the actual dry weight scanned per TEM grid surface ranged within 0.18 to 0.22 mg for 20 tissues.

Table 1. Chrysotile asbestos recovered in tissues of neonate baboons

	Tissue weight in mg deposited per grid		Loading factor, L		Fibers recovered 50 grid holes, F*		Concentration fibers/mg dry wt.**
	Test	Control	Test	Control	Test	Control	Test
Kidney cortex†	0.19	0.16	0.948	0.798	109	0[a]	7789
Lymph nodes	0.21	0.20	1.074	1.066	24	5[a]	1415
Spleen	0.20	0.20	1.010	1.000	15	2[b]	1030
Colon	0.20	0.20	0.991	1.019	12	2[b]	807
Esophagus	0.20	0.21	1.013	1.046	8	1[c]	553
Kidney medulla	0.19	0.18	0.940	0.910	4	0[c]	340
Stomach	0.20	0.21	0.977	1.051	2	1	82
Liver	0.20	0.18	0.980	0.890	1	0	82
Duodenum	0.20	0.22	0.996	1.086	3	3	-
Cecum	0.20	0.20	1.023	0.994	5	8	-
Heart	0.20	0.20	1.002	1.023	BDL††	0	BDL††

* Preparations from test and control kidney cortex were examined for 62 and 60 grid holes, respectively.

** Fibers/mg dry wt. = $\frac{F \times 80}{L}$. The values shown were after correction for control levels.

† Kidney cortex data was taken from Patel-Handlik, et al.[13]

†† Below Detection Limit

[a] $P \leq 0.001$, [b] $P=0.01$, [c] $P=0.05$ are for comparison between test and control tissues

DETECTION LIMIT

The detection limit of this analytical technique can be calculated by substituting values of F and L in the above equation (iii). If one fiber was found per 50 grid holes scanned and if L was one, the detection limit would be 80 F/mg dry weight. The detection limit can be improved by viewing more grid holes or by increasing V_2 and/or M, which in turn, may be limited by the amount of extraneous material on the grid surface to obstruct in the scanning under the TEM. The above detection limit is almost two times more sensitive than that obtained by Cunningham, et al.[17]

RESULTS AND DISCUSSION

Tissue preparative technique reported here for TEM analysis of asbestos fibers has the following merits:

1. It can be used for analysis of fresh, frozen or formalin preserved tissues, and fresh filtered urine.(15)

2. The sample suspension can be preserved by allowing the acetone to evaporate under cover and freezing it. Since leaching of asbestos metals is prevented during such preservation in the dry state, fresh grids can be prepared for future TEM analysis by resuspending the ash in acetone.

3. Since almost equal weights of the different tissues under analysis are deposited on each grid surface [by adjusting the dry weight of tissue times the volume of suspension on a grid (total volume of suspension)], the fiber count itself permits instant comparisons of the levels of fibers in the different tissues.

4. The detection limit is very good.

Two grids each prepared from the test and control kidney cortex tissues were coded and sent to another laboratory. On uncoding, the results on the fiber count were found to be remarkably close to those obtained at this laboratory indicating the reliability of this analytical technique. This technique differs from that used by Cunningham, et al [17,18] in two respects: tissue ash was solubilized in acetic acid instead of hydrochloric acid; and, the ash of the filter bearing the tissue ash was suspended in acetone instead of water for the purpose of transferring it onto a TEM grid.

Using the above technique grids were prepared for TEM analysis of eleven tissues each, of control and test neonate baboons. Chrysotile asbestos count and size data were obtained by examining 50 grid holes, 25 each on two grid preparations from each tissue.[1] Table 1 shows the results on the recovery of asbestos in eleven tissues of a test baboon on a gradient of high to low contents. In general, the number of fibers recovered in the tissues of control animal was low. Concentrations of fibers (F/mg dry weight) in the test tissues were calculated after correction for the fibers found in the respective tissues of the control neonate baboon. Retention of fibers in test cecum could not be evaluated because more fibers were recovered in control sample than in the test sample.

1. Sixty-two and 60 grid holes, respectively, were examined on the grids prepared from test and control kidney cortex tissues. Thanks are due to P.J. Clark and J.R. Millette of the Health Effects Research Laboratory, U.S. EPA, who were kind enough to provide the TEM scanning data of the test kidney cortex grids.

Each pair of test and control tissue data was compared using Welsh's t-test,[19] and $P \leq 0.005$ was interpreted as indicating significant difference. A highly significant difference ($P \leq 0.001$) was found between the paired means of fibers recovered in kidney cortex and in lymph nodes.

Welsh's t-test was applied after corrections for control values to compare the fiber levels among test organs. Figure 1 illustrates multicomparisons among concentrations of asbestos recovered in eight test tissues. The histogram permits to read the statistical difference at $P \leq 0.5$ in the fiber levels between any two tissues; for example, when tissue no. 1 or kidney cortex was compared individually with seven other tissues numbered as 2 thru 8 it indicated statistically significant excess of asbestos retention ($P \leq 0.001$). There was no significant difference between the fiber levels recovered in spleen and colon as shown by the absence of vertical bar for comparison between spleen and tissue no. 4.

For evaluation of the results of this research a level of $P \leq 0.005$ was considered as indicative of significant difference. The kidney cortex showed a highly significant difference ($P \leq 0.001$) from all other organs under analysis (Fig. 1). Also, a similar high P value was obtained when comparisons were made between the fiber levels in lymph nodes and kidney medulla, lymph nodes and stomach, lymph nodes and liver, and colon and liver. Differences were significant P=0.005) between the fiber levels in spleen and stomach, spleen and liver, and colon and stomach. Caution is required in drawing inferences from the results wherever the fiber counts are very low (see Table 1).

Nested analysis of variance was performed to test the particulate distribution on grids prepared from same sample. The difference between two populations (grids) was significant (P=0.005) when the fiber counts derived from all tissues were compared. Hence, comparisons were made using Welsh's t-test between the fiber counts recovered in 25 grid holes of two grids each for each of the ten tissues. Eight of the ten tissues did not show significant difference (P=0.005) between the replicate counts of two grids prepared from each. Fiber counts were significantly different between two grids prepared from each kidney cortex and spleen (P=0.005).

Tables 2-5 show fiber size and distribution of chrysotile fibers recovered in test tissues. The total number of fibers recovered in each tissue is also given in the Tables because the data are meaningful only when the number of fibers on which it is based is known. Results given in Tables 1-5 include the data on asbestos fibers recovered in the kidney cortex published recently.[13] The range in length of fibers was 0.16 μm to 35.0 μm among all the tissues, and was 0.16 μm to 9.30 μm among five gastrointestinal tract organs. The longest fiber was recovered from test kidney cortex. The diameters ranged from 0.01 μm to 1.50 μm among all the tissues and 0.02 μm to 0.16 μm among five gastrointestinal tract organs. This indicated that fibers retained by gastrointestinal tract were of smaller size in relation to other regions. Wide ranges of length, diameter, aspect ratio, and recovery of bundles and clusters in all organs (including colon wherein several bundles of degraded morphology, class C, were recovered) suggest that the processes of penetration and migration occur for all sizes. A cluster of fibers recovered in test

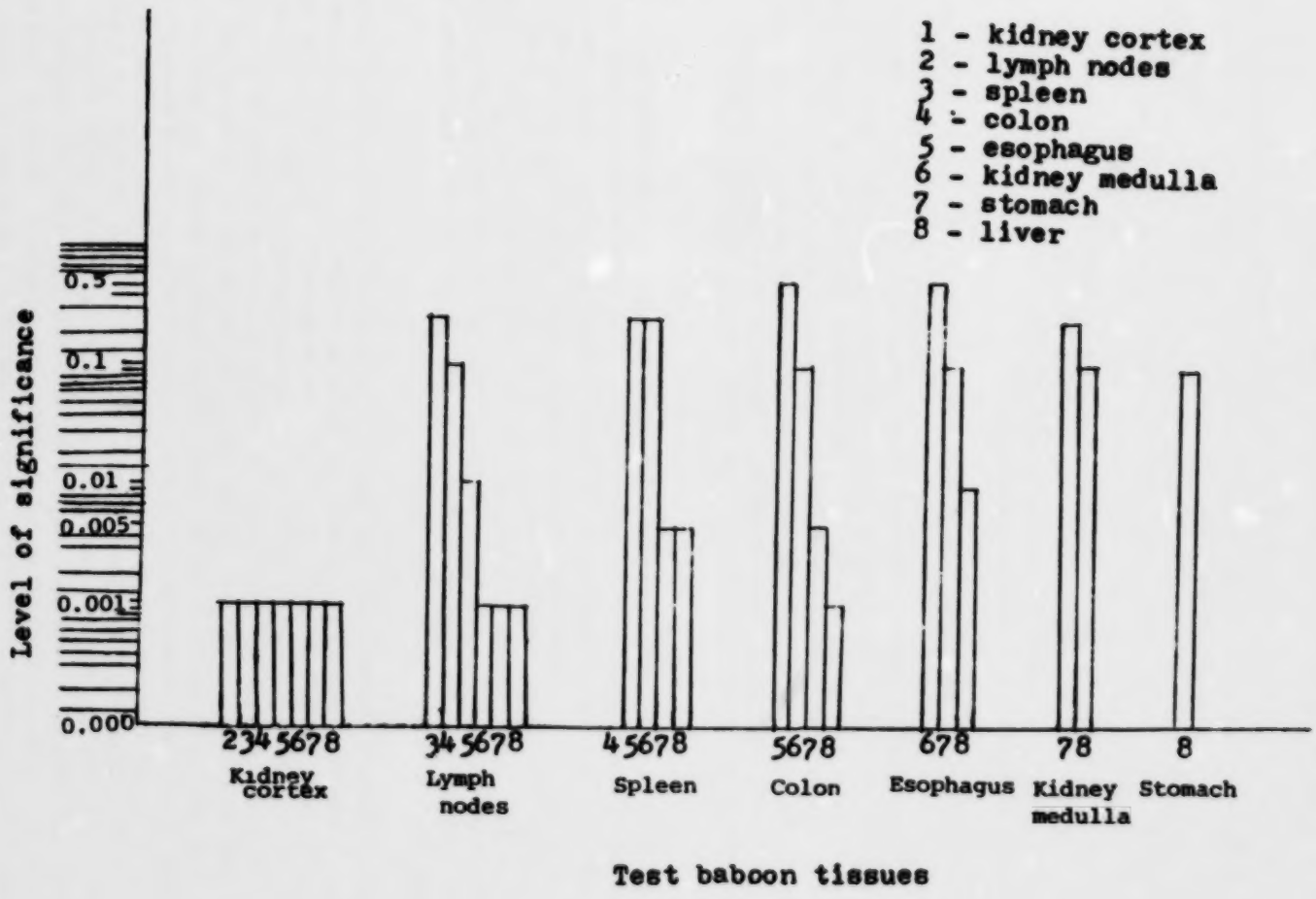

Fig. 1. Statistical difference in the fiber levels retained by test baboon tissues - comparisons between each two tissues.

spleen is illustrated in Fig. 2. Another cluster recovered in test spleen was about three times as big as that shown in Fig. 2.

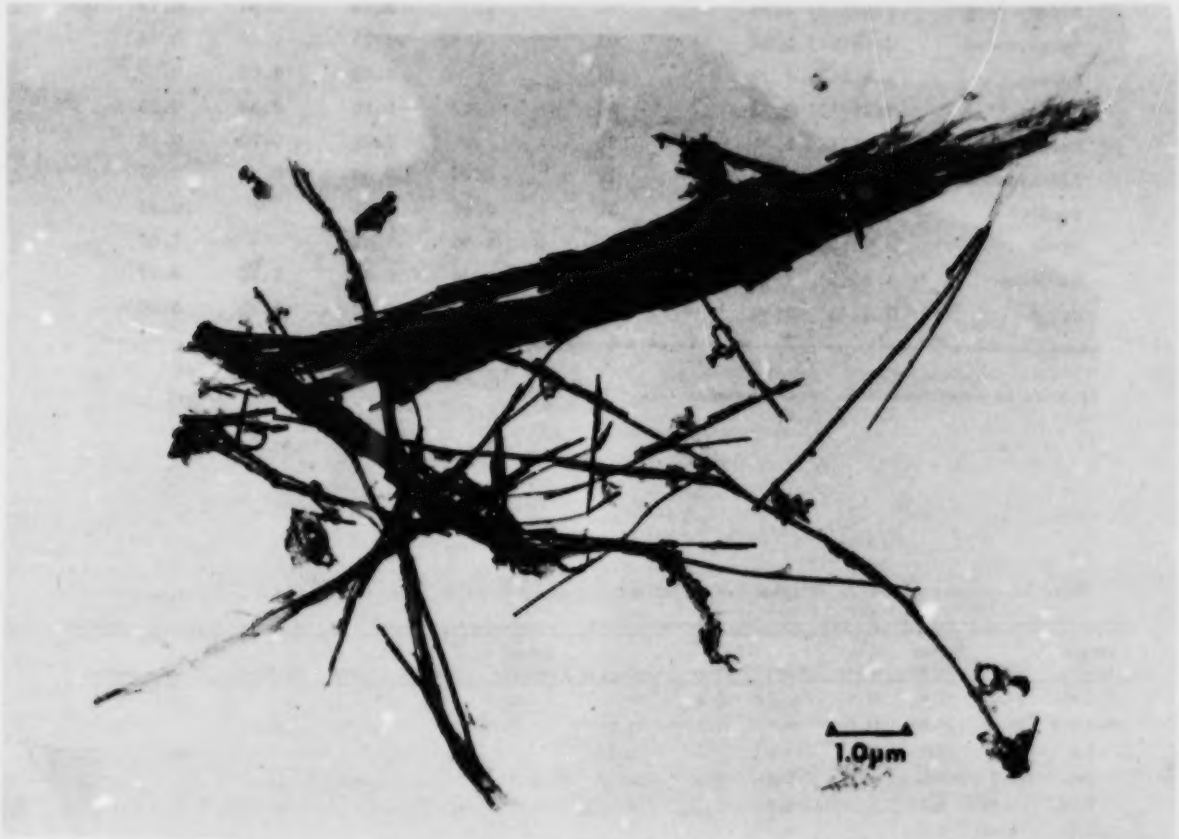

Fig. 2. A cluster of chrysotile asbestos fibers recovered in the spleen of test neonate baboon. Bar length = 1.0 µm

Frequency of distribution of fiber lengths (Table 3) of retained fibers shows a general pattern almost comparable to the distribution frequency of administered fibers.[20]

Table 2. Length data in μm of chrysotile fibers recovered in test baboon tissues.

Tissue	Mean	Standard deviation	Coefficient of variation	Maximum	Minimum	Median	Geometric Mean
Kidney cortex	1.7(109)*	4.38	257	35	0.24	0.50	0.73
Lymph nodes	0.86(24)	0.83	97	3.30	0.28	0.56	0.65
Spleen	1.87(15)	3.29	174	12.80	0.20	0.60	0.87
Colon	0.33(12)	0.22	64	0.80	0.16	0.14	0.29
Esophagus	1.16(8)	1.04	90	2.84	0.22	0.70	0.76
Kidney medulla	0.42(4)	0.10	22	0.50	0.30	0.50	0.42
Stomach	0.18(2)	0.06	16	0.40	0.32	-	0.36
Liver	4.80(1)	0.00	0	4.80	4.80	-	4.80
Duodenum	4.20(3)	4.54	108	9.30	0.60	2.70	2.47
Cecum	0.52(5)	0.50	94	1.40	0.24	0.28	0.40

*Figures in parentheses were total of number fibers recovered

Table 3. Percent frequency of fiber lengths of UICC standard Canadian chrysotile and chrysotile fibers recovered in test baboon tissues

Length in μm	UICC*	Kidney cortex	Lymph nodes	Spleen	Colon	Esophagus	Kidney medulla	Stomach	Liver	Duodenum	Cecum	Heart
0.2-05	30.6	37.6	37.5	40.0	83.3	50	100	100			60	
0.5-1.0	33.4	26.6	37.5	26.7	16.7	12.5				33.3		
1-2	19.8	23.0	12.5	13.3		1.5					30	
2-5	13.2	5.8	12.5	6.7		35			100	33.3		
5-10	1.76	3.7		6.7						33.3		
10.25	0.93	2.7		6.7								
25-50	0.24	0.9										
50-100	0.07											
Total No. of fibers recovered†	109	24	15	12	8	4	2	1	3	5	BDL‡	

* Data from Rendall, R.E.G.(20)

† Per cent frequency data should be considered in the light of total number of fibers recovered.

‡ Below detection limit

Table 4. Fiber diameter data of chrysotile asbestos recovered in test baboon tissues

Tissue	Mean	Standard deviation	Coefficient of variation	Maximum	Minimum	Median	Geometric mean
Kidney cortex	0.04(109)*	0.04	100	0.34	0.03	0.03	0.04
Lymph nodes	0.08(24)	0.08	107	0.32	0.01	0.04	0.05
Spleen	0.15(15)	0.37	241	1.50	0.02	0.04	0.06
Colon	0.05(12)	0.04	74	0.14	0.02	0.04	0.04
Esophagus	0.07(8)	0.02	31	0.11	0.03	0.08	0.07
Kidney medulla	0.05(4)	0.02	40	0.08	0.04	0.04	0.05
Stomach	0.08(2)	0.06	71	0.12	0.04	-	0.07
Liver	0.12(1)	0.00	0	0.12	0.12	-	0.12
Duodenum	0.08(3)	0.074	96	0.16	0,02	0.05	0.05
Cecum	0.06(5)	0.02	34	0.08	0.04	0.08	0.06

*Figures in parentheses were total number of fibers recovered

Table 5. Type and size of fibers recovered in test baboon tissues

Tissue		Kidney cortex	Lymph nodes	Spleen	Colon	Esophagus	Kidney medulla	Stomach	Liver	Duodenum	Cecum
Recovery of fibers	Classes* A+B	109	24	15	12	8	4	2	1	3	5
	Class C	0	2	0	21	0	0	0	0	1	3
	Per cent burdles/ clusters	Not recorded	29	33	0	37.5	25	50	100	66	20
Aspect Ratio	Mean	39.50	22.58	19.91	7.85	15.57	8.95	6.33	40.0	108.25	7.85
	Max.	800.0	104.0	70.0	12.0	35.50	12.50	10.0	40.0	186.0	7.50
	Min.	5.0	1.75	7.5	3.75	3.60	6.25	2.67	40.0	3.75	3.25

*Fibers graded as Classes A and B were included to obtain the fiber concentration in the tissues. Fibers of Class C indicated the degradation of fibers making it difficult to confirm their chrysotile identity.

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